Cross-Reactivity of Three Recombinant Insulin Analogs with Five Commercial Insulin Immunoassays

To the Editor:

Several new insulin analogs that are prepared with recombinant DNA technology are available for clinical use [for a recent review, see Ref. *(1)*]. These agents have altered pharmacokinetics compared with regular human insulin. Insulin aspart (NovoLog™; Novo Nordisk Pharmaceuticals) is homologous with regular human insulin except for a single substitution of aspartic acid for proline at position B28. This single substitution reduces the molecule's tendency to form hexamers. Therefore, insulin aspart is absorbed more rapidly after subcutaneous injection and has both a faster onset of action and a shorter duration of action than regular insulin. A second short-acting recombinant insulin is insulin lispro (Humalog®; Eli Lilly and Company), which is a human insulin analog

created by reversing the amino acids at positions 28 (Pro to Lys) and 29 (Lys to Pro) of the B chain. It is absorbed more rapidly than regular human insulin when administered subcutaneously and has a shorter duration of action.

A third recombinant insulin that is longer acting is insulin glargine (Lantus®; Aventis Pharmaceuticals), which differs from regular human insulin by the substitution of glycine for asparagine at position A21 and by the addition of two arginine residues to the COOH terminus of the B chain. These modifications lead to a slower, more prolonged absorption than regular insulin and a relatively stable concentration–time profile over 24 h. Insulin glargine is partially metabolized at the COOH terminus of the B chain in the subcutaneous depot to form two active metabolites, M1 and M2, with in vitro activity similar to that of insulin. Metabolite M1 is 21^{A}-Gly-insulin, and metabolite M2 is 21^{A}-Gly-des-30^{B}-Thr-insulin.

One obvious question with these recombinant preparations is whether they are detectable by immunoassay. Immunoassays have been developed specifically for pharmacokinetic studies of insulin analogs. A sensitive RIA that is specific for insulin lispro and an ELISA that is specific for insulin aspart have been developed (*2*, *3*). A chemiluminescent enzyme immunoassay that can quantify human insulin, proinsulin, despentapeptide insulin, porcine insulin, and insulin lispro with comparable cross-reactivity has also been described (*4*). Two insulin assays, one that can specifically quantify human insulin and a second with which human insulin, insulin aspart, and insulin lispro cross-react equally, have been used to estimate the concentration of insulin aspart or lispro by subtraction (*5*). All of these assays are used primarily for research and are not commonly used in clinical laboratories. Two reports have appeared in this journal describing the use of a commercial RIA for quantifying insulin lispro (*6*, *7*). Only one study to date has examined the cross-reactivity of insulin lispro with an automated insulin assay on a multichannel, random-access analyzer (*8*). These authors found that the cross-reactivity of insulin lispro in the Elecsys insulin assay was <0.02%. The goal of this study was to quantify the cross-reactivity of insulin aspart, insulin glargine, and insulin lispro with several commercial insulin assays to determine which might be specific for human insulin and which might show cross-reactivity with the insulin analogs. Four of the five insulin assays were performed on automated, multichannel, random-access analyzers that are available in clinical laboratories.

We obtained vials of each of the three insulin analogs, with a nominal concentration of 100 IU/mL and suitable for injection, from our hospital pharmacy. Each was diluted volumetrically with 60 g/L aqueous bovine serum albumin to final insulin concentrations of 30, 100, 300, and 1000 mIU/L. All dilutions of each insulin preparation were analyzed in duplicate, and the percentage cross-reactivity was calculated from the ratio of the measured and nominal concentration. Measurements were made on an Access analyzer (Beckman Coulter), an Advia Centaur analyzer (Bayer Diagnostics), an E170 analyzer (Roche Diagnostics), and an IMMULITE 2000 analyzer (Diagnostics Products Corporation), using the manufacturers' reagents according to the instructions. Two lots of reagent for the IMMULITE 2000 were examined: lot 122, which is the old formulation, and lot 151, which is a new formulation, presumably with a change in one or both antibodies. In addition, a manual assay, Coat-A-Count (Diagnostic Products Corporation), was also used for testing.

A summary of the recoveries observed for each combination of analog and assay method is presented in Table 1. All analogs had an equivalent cross-reactivity of 80% with the Access method. This may represent cross-reactivity that is truly <100%, or assay calibration may not exactly match the nominal concentration of each insulin analog. Insulin lispro demonstrated 90% cross-reactivity on the Advia Centaur, whereas insulin aspart and glargine had mean cross-reactivities of 126% and 143%, respectively. Each of the analogs had 35–45% cross-reactivity with the Coat-A-Count assay. The E170 method did not detect any of the three analogs even at the highest concentrations tested. Both insulin aspart and lispro had ~28% cross-

Table 1. Cross-reactivities of insulin analogs.

	Cross-reactivity, %					
					IMMULITE 2000	
Analog and concentration	Access	Advia Centaur	Coat-A-Count	E170	Lot 122	Lot 151
Insulin aspart						
30 mIU/L	85.3	120	36.7	<0.7	14.7	9.3
100 mIU/L	80.0	124	57.0	<0.2	17.4	5.4
300 mIU/L	84.3	135	54.7	<0.07	40.7	8.0
1000 mIU/L	77.1	125	34.4	<0.02	40.7	13.0
Mean	81.7	126	45.7		28.4	8.9
Insulin glargine						
30 mIU/L	91.7	129	32.0	<0.7	<6.7	8.3
100 mIU/L	85.0	140	46.0	<0.2	3.4	2.8
300 mIU/L	78.7	152	35.7	<0.07	11.1	1.6
1000 mIU/L	79.7	150	27.1	<0.02	13.2	1.8
Mean	83.8	143	35.2		9.2	3.6
Insulin lispro						
30 mIU/L	78.7	86.7	37.0	<0.7	14.7	10.3
100 mIU/L	77.0	89.0	52.0	<0.2	18.0	6.3
300 mIU/L	79.3	92.3	49.7	<0.07	42.3	8.4
1000 mIU/L	80.2	89.2	33.4	<0.02	39.3	12.3
Mean	78.8	89.3	43.0		28.6	9.3

reactivity with the old formulation assay on the IMMULITE 2000, and insulin glargine had 9% cross-reactivity. Both insulin aspart and lispro had 9% cross-reactivity with the new formulation assay on the IMMULITE 2000, and insulin glargine had ~4% cross-reactivity.

The large variability in insulin analog cross-reactivities with different commercial assays is noteworthy. The least cross-reactivity was observed for the E170 method, which had a cross-reactivity of <0.02% for all three analogs. Our result of no detectable cross-reactivity for insulin lispro is in agreement with a previous report *(8)*. This suggests that one antibody used in this assay recognizes either an epitope that includes A21 or an epitope that includes B28 and the COOH terminus of the B chain. Substitutions at one of these positions abolish immunoreactivity.

The Access assay demonstrated similar recoveries for all three analogs that were close to the nominal concentrations. It seems plausible that neither antibody used in this assay recognizes an epitope close to A21 or B28/COOH terminus because neither substitution affected insulin recovery. The highest cross-reactivity was for insulin glargine on the Advia Centaur, with 152% cross-reactivity at 300 mIU/L. This method also showed a cross-reactivity of 126% for insulin aspart. The substitutions at A21 and B28 enhance the binding of the antibodies used in this assay. The Coat-a-Count and IMMULITE 2000 insulin assays, which are from the same manufacturer, apparently use different antibodies. The reformulation of the IMMULITE 2000 assay led to a decreased cross-reactivity with all three analogs.

It is also interesting that the cross-reactivity can be concentration dependent. This was most notable for the IMMULITE 2000 assay. Previous studies of the Coat-a-Count assay with insulin lispro found a cross-reactivity of ~100% with one lot of RIA tubes and a cross-reactivity of 186% with a different lot *(7)*. We found a mean cross-reactivity of 43%, suggesting that there may have been another change in the antibodies used compared with earlier studies.

It may be important for clinicians to be aware of the cross-reactivities of various insulin analogs with the assay(s) used by an individual clinical laboratory. Unfortunately, the package inserts for the assays we evaluated did not include this information. An update to the package insert about cross-reactivity with insulin analogs would also be desirable if an assay is modified, particularly if an antibody were changed. Another interesting point from this study is that a combination of commercial assays can be used to estimate the concentrations of these insulin analogs in the presence of human insulin. For example, the E170 measures only human insulin, whereas the Access insulin assay measures all three analogs with a cross-reactivity of ~80%. Use of a combination of these two assays should make it possible to estimate the concentration of any of these three insulin analogs in the presence of human insulin.

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